

AMENDED CLAIMS (Article 34 PCT)

1. A method for isolating plasmid DNA from a DNA containing material which comprises plasmid DNA and genomic DNA, comprising the steps of:

- 5 (i) extracting the plasmid DNA into butanol by mixing the material with butanol, a chaotrope, and water under conditions to denature the genomic DNA; and
- (ii) recovering the plasmid DNA from the butanol.

10 2. The method of claim 1, wherein the conditions to denature the DNA comprise basic conditions or a temperature of at least 65°C.

3. The method of claim 2, wherein the conditions to denature the DNA comprise basic conditions in which a base is present.

15 4. The method of any preceding claim, wherein the butanol is n-butanol, 2-methylpropanol, or butan-2-ol.

20 5. The method of any preceding claim, wherein the chaotrope is selected from the group consisting of guanidine hydrochloride, guanidine thiocyanate, sodium perchlorate, and mixtures thereof.

6. The method of claim 6, wherein the chaotrope comprises guanidine hydrochloride.

25 7. The method of any one of claims 3 to 6, wherein the base comprises a hydroxide.

8. The method of claim 7, wherein the hydroxide comprises sodium hydroxide.

30 9. The method of any one of claims 3 to 8, wherein the butanol, the chaotrope, the base and the water are combined to form an extraction mixture, and extraction step (i) comprises mixing the extraction mixture with the plasmid DNA-containing material.

10 The method of any preceding claim, wherein the amount of butanol is in the range from 20 to 70% based on the volume of the combination of butanol, chaotrope and water.

11. The method of claim 10, wherein the amount of the butanol is in the range from 35 to 50%.

12. The method of claim 11, wherein the amount of the butanol is about 42%.

Sub 5
a3 13. The method of any preceding claim, wherein the chaotrope is present at a concentration of from 0.7M to 1.2M based on the combination of butanol, chaotrope and water.

14. The method of claim 13, wherein the concentration of the chaotrope is about 0.9M.

Sub 10
a4 15. The method of any preceding claim, wherein the recovery step (ii) comprises mixing the DNA-containing butanol phase with a precipitating agent that can precipitate the plasmid DNA from the butanol, and separating the precipitated plasmid DNA from the butanol.

16. The method of claim 15, wherein the recovery step (ii) further comprises a washing step
15 in which the precipitated plasmid DNA is washed.

17. The method of claim 15 or claim 16, wherein the precipitating agent comprises an alcohol.

18. The method of claim 17, wherein the alcohol is ethanol.

20 Sub 20
a5 19. The method of any one of claims 15 to 18, wherein the precipitating agent further comprises an acetate salt.

20. The method of claim 19, wherein the acetate salt comprises sodium acetate.

25 Sub 25
a6 21. The method of any preceding claim, which further comprises a step of separating the organic and aqueous phases of step (i) prior to recovering the plasmid DNA.

22. The method of claim 21, wherein the step of separating the organic and aqueous phases
30 further comprises centrifugation of the mixture formed in step (i) to facilitate separation of the mixture into the organic and aqueous phases.

Sub 30
a7 23. The method of any preceding claim, wherein the DNA-containing material comprises a lysed or unlysed bacterial culture.

24. An extraction mixture for selectively extracting plasmid DNA from a DNA-containing material, which extraction mixture comprises butanol, a chaotrope, and water.

25. The extraction mixture of claim 24, which further comprises a base.

26. The extraction mixture of claim 25, wherein the base comprises a hydroxide.

27. The extraction mixture of claim 26, wherein the hydroxide comprises sodium hydroxide.

28. The extraction mixture of any one of claims 24 to 27, wherein the butanol is n-butanol, 2-methylpropanol, or butan-2-ol.

29. The extraction mixture of any one of claims 24 to 28, wherein the butanol constitutes from 20 to 70% based on the volume of the extraction mixture.

30. The extraction mixture of claim 29, wherein the butanol constitutes from 35 to 50 % of the extraction mixture.

31. The extraction mixture of claim 30, wherein the butanol constitutes about 42% of the extraction mixture.

32. The extraction mixture of any one of claims 24 to 31, wherein the chaotrope is selected from the group consisting of guanidine hydrochloride, guanidine thiocyanate, sodium perchlorate, and mixtures thereof.

33. The extraction mixture of claim 32, wherein the chaotrope comprises guanidine hydrochloride.

34. The extraction mixture of any one of claims 24 to 33, wherein the concentration of chaotrope in the extraction mixture is from 0.7M to 1.2M.

35. The extraction mixture of claim 34, wherein the concentration of the chaotrope in the extraction mixture is about 0.9M.